1. Introduction

Olink® IMMUNE RESPONSE is a reagent kit measuring 92 immune response-related human protein biomarkers simultaneously using just 1 µL of serum, plasma or other human sample type. The analytical performance of the product has been carefully validated and the results are presented in this document. Please note that when a new panel is developed, both the individual assays and 92-plex panel as a whole are subject to our thorough validation procedure. If individual assays are subsequently improved or one or more assays are replaced in later versions of the panel, focus is placed on thoroughly validating the individual assays in question.

1.1 TECHNOLOGY

The Olink reagents are based on the Proximity Extension Assay (PEA) technology[1-2], where 92 oligonucleotide labeled antibody probe pairs are allowed to bind to their respective target proteins, if present in the sample. A PCR reporter sequence is formed by a proximity-dependent DNA polymerization event. This is then amplified, and subsequently detected and quantified using real-time PCR. The assay is performed in a homogeneous 96-well format without any need for washing steps, see Figure 1.

1.2 QUALITY CONTROLS

Internal and external controls have been developed by Olink for data normalization and quality control purposes. These controls are designed to enable monitoring of the technical assay performance, as well as the quality of individual samples, and provide information at each step of the Olink protocol (see Figure 1). The internal controls are added to each sample and include two Immunoassay controls, one Extension control and one Detection control. The Immunoassay controls (two non-human proteins) monitor all three steps starting with the immunoreaction. The Extension Control (an antibody linked to two matched oligonucleotides) monitors the extension and readout steps independent of antigen binding, and is used for data normalization across samples. Finally, the Detection control (a synthetic double-stranded template) monitors the readout step. Samples for which one or more of the internal control values deviate from a pre-determined range will be flagged and may be removed before statistical analysis. An external inter-plate control (IPC), is included on each plate and is used in a second normalization step. This control is made up of a pool of probes similar to the Extension control (Ext Ctrl), but generated with 92 matching oligonucleotide pairs. This improves inter-assay precision and allows for optimal comparison of data derived from multiple runs. The term "Normalized Protein eXpression (NPX)" refers to normalized data as described above.

1.3 DATA ANALYSIS

Data analysis is performed by employing a pre-processing normalization procedure. For each sample and data point, the corresponding Cq-value for the Extension control is substracted, thereby normalizing for technical variation within one run. Normalization between runs is then performed for each assay by substracting the corresponding dCq-value for the Interplate Control (IPC) from the dCq-values generated. In the final step of the pre-processing procedure the values are set relative to a correction factor determined by Olink. The Normalized Protein eXpression (NPX) unit is generated on a log2 scale where a larger number represents a higher protein level in the sample, typically with the background level at around zero. Linearization of data is performed by the mathematical operation $2^{\wedge NPX}$. Coefficient of variation (CV) calculations are performed on linearized values.

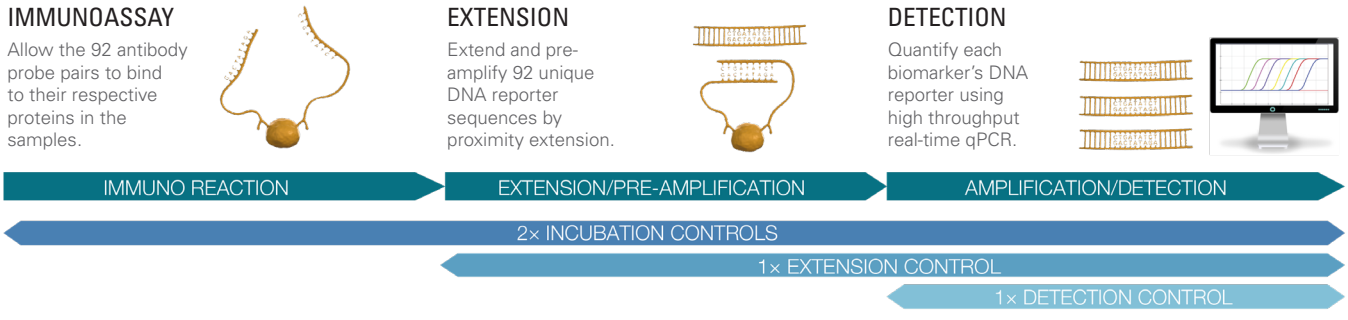

Fig 1. Olink assay procedure (above) and controls (below). The internal controls enables monitoring of the three core steps in the Olink assay and used for quality control and data normalization. Read out is performed by using the Fluidigm® Biomark™ or the Fluidigm® Biomark™ HD system.

2. Performance characteristics

2.1 SAMPLE TYPES

The ability to use different sample types was evaluated with Olink IMMUNE RESPONSE by collecting matched serum, EDTA, acid citrate dextrose (ACD), and sodium heparin plasma samples from 4 healthy individuals. Table 1 summarizes response values for 22 normal EDTA plasma samples expressed in NPX, as well as relative differences compared to EDTA plasma. Variations observed between responses in heparin, citrate plasma and serum, as compared to EDTA plasma, were generally small, and all assays will therefore function without limitation in these sample types.

2.2 ANALYTICAL MEASUREMENT

NOTE: *The technical performance data based on in vitro assays using recombinant antigen must* **NOT** *be used to derive actual concentrations of native proteins in biological samples from the relative quantification NPX data that is obtained from an Olink assay.*

DETECTION LIMIT

Calibrator curves were determined for 91 out of 92 biomarkers simultaneously in a multiplex format. In cases where no suitable antigen was available, no calibrator data is presented. Limit of detection (LOD) was defined as 3 standard deviations above background, and reported in pg/mL, see Table 1.

HIGH DOSE HOOK EFFECT

The high dose hook effect is seen when there is an antigen excess relative to the reagent antibodies, resulting in falsely lower values. In such cases, a significantly lower value can be reported that can lead to misinterpretation of results. Therefore, the hook threshold was determined for each analyte and reported in pg/mL, see Table 1.

MEASURING RANGE

The analytical measuring range was defined by the lower limit of quantification (LLOQ) and upper limit of quantification (ULOQ) and reported in pg/mL. Quantification limits of LLOQ and ULOQ were calculated with the following trueness and precision criteria; relative error ≤ 30% and CV ≤ 30%, of back-calculated values, respectively. Measuring ranges are presented in Table 1, ordered by LLOQ and displayed on a log10 scale.

Example calibrator curves showing the measuring ranges for selected representative assays are shown in Figure 2. The overall distribution of measuring ranges for the assays with available recombinant antigens is shown in Figure 3. Separate calibrator curves established for each assay may be viewed at www.olink.com.

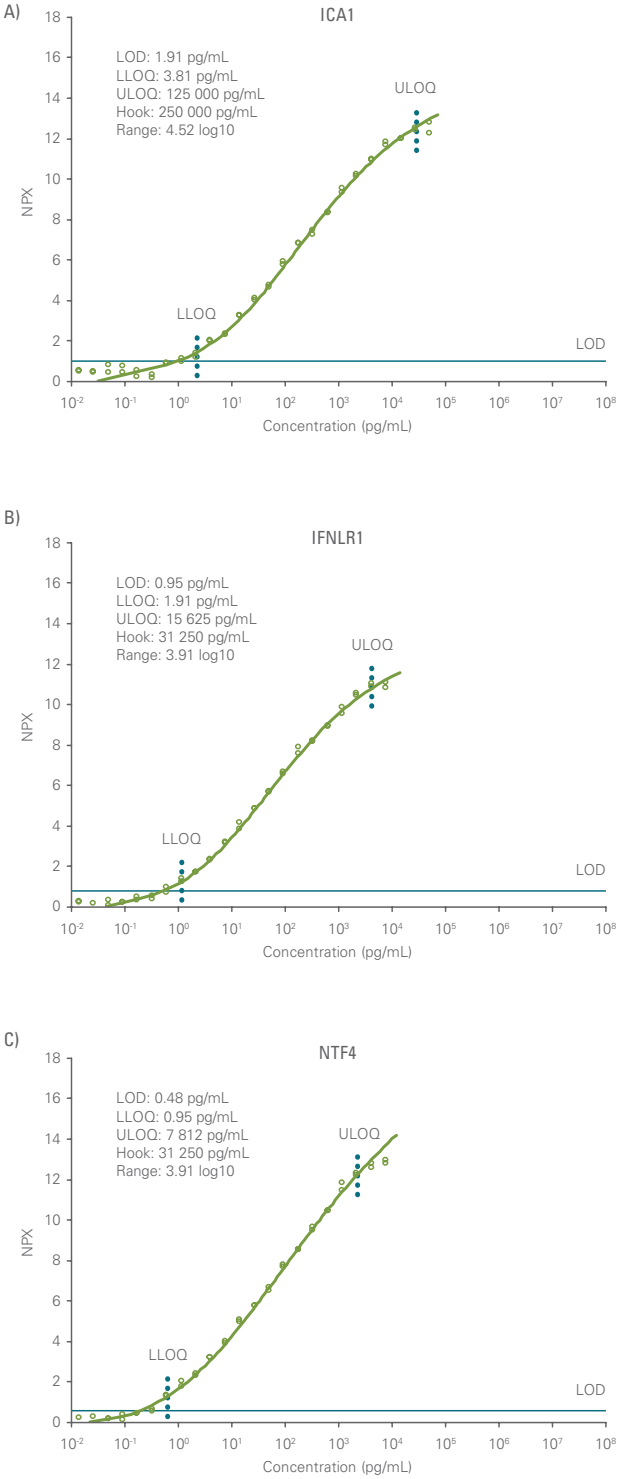

Fig 2. Calibrator curves for representative assays using a 4-parameter curve fitting model.

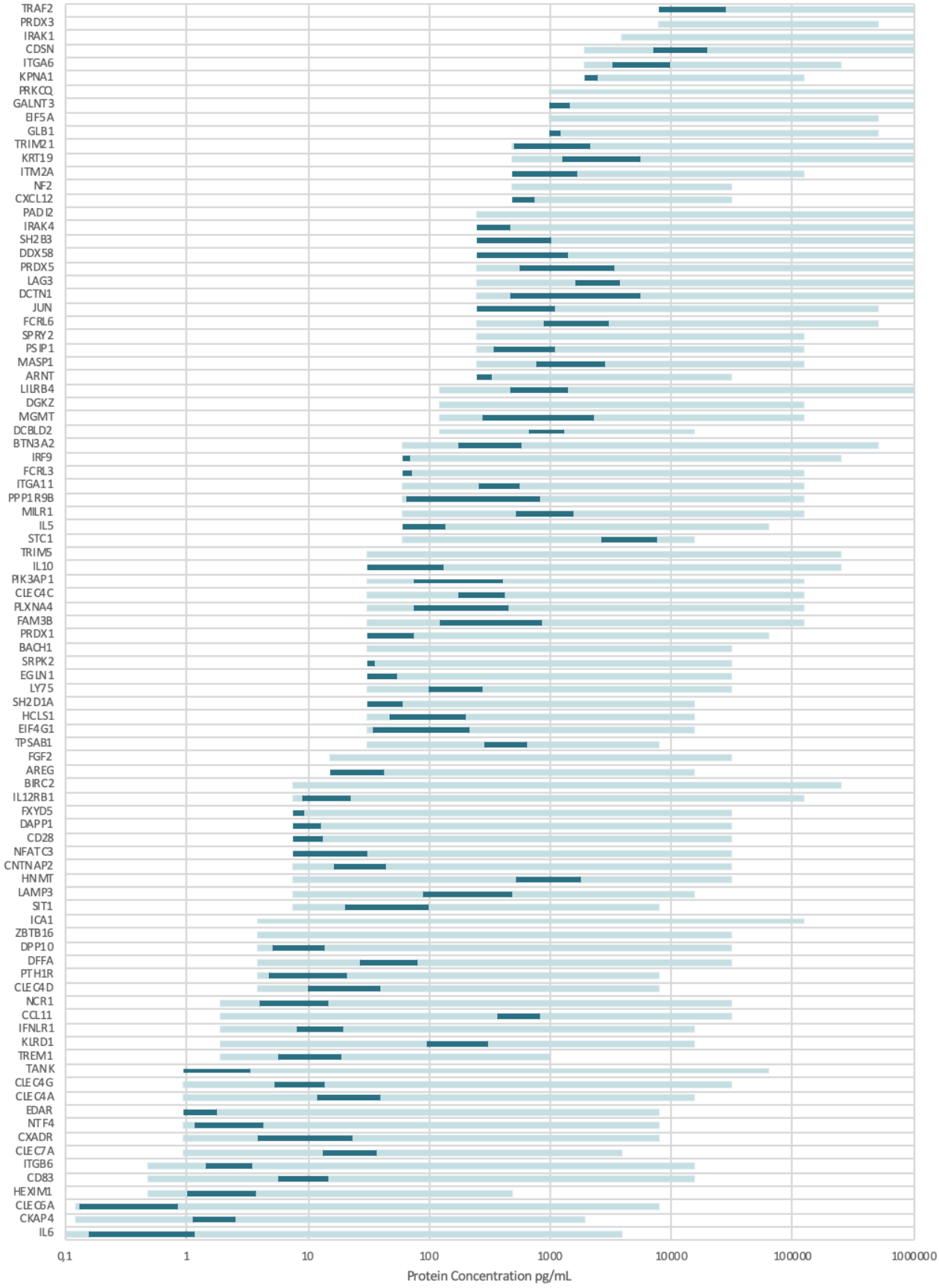

Fig 3. Distribution of analytical measuring range, defined by the lower and upper limits of quantification (LLOQ-ULOQ), and normal plasma levels where data is available (dark blue bars) for 91 out of 92 analytes.

Table 1. Sample Types; Normalized Protein eXpression (NPX), Endogenous Interference, Analytical Measurement; Limit of Detection (LOD), Lower Limit of Quantification (LLOQ), Upper Limit of Quantification (ULOQ), High Dose Effect (Hook), Range and Precision indicative of assay performance are shown for 92 analytes. Not available, NA

		Sample types						Endogenous interference	Analytical measurement					Precision	
		Normal plasma levels (NPX)			Relative to EDTA plasma (%)			(mg/mL)	pg/mL				log10	% CV	
Target	UniProt No	10th %tile	Median	90th %tile	ACD	Heparin	Serum	Hemolysate	LOD	LLOQ	ULOQ	Hook	Range	Intra	Inter
Allergin-1 (MILR1)	Q7Z6M3	2.5	3.3	3.9	125	97	116	0.9	61	61	125 000	500 000	3.3	16	21
Amphiregulin (AREG)	P15514	1.6	2.0	2.9	100	95	99	15	1.91	15	15 625	31 250	3.0	6	12
Aryl hydrocarbon receptor nuclear translocator (ARNT protein) (ARNT)	P27540	NA	NA	1.1	NA	NA	NA	15	244	244	31 250	125 000	2.1	10	18
Baculoviral IAP repeat-containing protein 2 (BIRC2)	Q13490	NA	0.2	0.5	NA	NA	NA	15	7.63	7.63	250 000	250 000	4.5	8	17
Beta-galactosidase (GLB1)	P16278	0.6	0.9	1.3	163	98	147	15	488	976	500 000	1 000 000	2.7	7	21
Butyrophilin subfamily 3 member A2 (BTN3A2)	P78410	1.4	1.9	2.4	129	96	122	7.5	61	61	500 000	1 000 000	3.9	8	16
CD83 antigen (hCD83) (CD83)	Q01151	2.7	3.1	3.8	114	94	111	15	0.48	0.48	15 625	15 625	4.5	8	16
Contactin-associated protein-like 2 (CNTNAP2)	Q9UHC6	1.6	2.1	2.4	124	94	118	15	7.6	7.63	31 250	125 000	3.6	9	18
Corneodesmosin (CDSN)	Q15517	3	3.7	4.4	114	92	120	0.9	976	1953	1 000 000	1 000 000	2.7	10	19
Corticosteroid 11-beta-dehydrogenase isozyme 1 (HSD11B1)	P28845	2.4	3.1	3.8	122	93	111	0.5	NA	NA	NA	NA	NA	7	15
Coxsackievirus and adenovirus receptor (CAR) (CXADR)	P78310	1.4	1.9	2.9	120	96	109	15	0.95	0.95	7 812	7 812	3.9	7	17
C-type lectin domain family 4 member A (CLEC4A)	Q9UMR7	3.2	3.8	4.7	131	97	114	15	0.9	0.95	15 625	31 250	4.2	7	15
C-type lectin domain family 4 member C (CLEC4C)	Q8WTT0	2.4	2.9	3.5	116	92	109	15	31	31	125 000	500 000	3.6	9	16
C-type lectin domain family 4 member D (CLEC4D)	Q8WXI8	1.7	2.8	3.4	202	98	165	15	0.95	3.81	7 812	31 250	3.3	8	16
C-type lectin domain family 4 member G (CLEC4G)	Q6UXB4	2.8	3.2	3.8	115	92	112	15	0.9	0.95	31 250	62 500	4.5	9	19
C-type lectin domain family 6 member A (CLEC6A)	Q6EIG7	1.1	1.9	3	146	90	138	15	0.12	0.12	7 812	31 250	4.8	9	18
C-type lectin domain family 7 member A (CLEC7A)	Q9BXN2	2.9	3.8	4.2	125	98	118	1.9	0.95	0.95	3 906	7 812	3.6	7	16
Cytoskeleton-associated protein 4 (CKAP4)	Q07065	4.5	4.9	5.4	147	95	120	3.8	0.12	0.12	1 953	7 812	4.2	7	17
Diacylglycerol kinase zeta (DAG kinase zeta) (DGKZ)	Q13574	NA	NA	0.3	NA	NA	NA	15	61	122	125 000	500 000	3.0	11	19
Discoidin, CUB and LCCL domain-containing protein 2 (DCBLD2)	Q96PD2	2.7	3.1	3.6	120	91	113	1.9	61	122	15 625	125 000	2.1	8	16
DNA fragmentation factor subunit alpha (DFFA)	O00273	2.5	3.1	3.8	115	96	111	15	1.91	3.81	31 250	125 000	3.9	8	16
Dual adapter for phosphotyrosine and 3-phosphotyrosine and 3-phosphoinositide (hDAPP1) (DAPP1)	Q9UN19	0.4	1.3	2.8	NA	94	NA	15	1.91	7.63	31 250	125 000	3.6	10	12
Dynactin subunit 1 (DCTN1)	Q14203	1.2	2.4	3.4	193	92	179	0.5	122	244	1 000 000	1 000 000	3.6	9	15
E3 ubiquitin-protein ligase TRIM21 (TRIM21)	P19474	1.2	1.8	2.3	97	95	86	15	122	488	1 000 000	1 000 000	3.3	9	18
Egl nine homolog 1 (EGLN1)	Q9GZT9	0.7	1.3	2.1	159	91	153	15	31	31	31 250	62 500	3.0	10	12
Eotaxin (CCL11)	P51671	7.2	7.8	8.4	117	98	114	15	1.91	1.91	31 250	31 250	4.2	7	13
Eukaryotic translation initiation factor 4 gamma 1 (eIF-4-gamma 1) (EIF4G1)	Q04637	1.2	2.4	3.6	48	94	46	15	15	31	15 625	62 500	2.7	7	14
Eukaryotic translation initiation factor 5A-1 (eIF-5A-1) (EIF5A)	P63241	NA	NA	0.7	NA	NA	NA	0.5	488	976	500 000	1 000 000	2.7	11	17
Fc receptor-like protein 3 (FcR-like protein 3) (FCRL3)	Q96P31	0.3	0.9	1.4	111	98	104	15	31	61	125 000	250 000	3.3	7	18
Fc receptor-like protein 6 (FcR-like protein 6) (FCRL6)	Q6DN72	2.2	3.1	3.6	116	91	107	15	122	244	500 000	1 000 000	3.3	8	14
Fibroblast growth factor 2 (FGF2)	P09038	NA	NA	0.7	NA	NA	NA	3.8	7.63	15	31 250	500 000	3.3	8	16
FXYD domain-containing ion transport regulator 5 (FXYD5)	Q96DB9	NA	NA	0.5	NA	NA	NA	15	7.63	7.63	31 250	62 500	3.6	10	16
Hematopoietic lineage cell-specific protein (HCLS1)	P14317	1.5	2.8	3.9	140	91	110	15	31	31	15 625	31 250	2.7	8	14
Histamine N-methyltransferase (HNMT)	P50135	8.2	9.1	10.3	109	100	101	15	3.81	7.63	31 250	62 500	3.6	9	15
Importin subunit alpha-5 (KPNA1)	P52294	NA	NA	1.9	NA	NA	NA	15	976	1953	125 000	250 000	1.8	10	15
Inactive dipeptidyl peptidase 10 (DPP10)	Q8N608	1.5	1.9	2.3	112	91	111	0.5	0.95	3.81	31 250	62 500	3.9	8	16
Integral membrane protein 2A (ITM2A)	O43736	1.3	1.6	3.2	81	93	77	15	122	488	125 000	1 000 000	2.4	25	38
Integrin alpha-11 (ITGA11)	Q9UKX5	2.9	3.4	3.8	77	92	73	3.8	31	61	125 000	1 000 000	3.3	8	19
Integrin alpha-6 (ITGA6)	P23229	1.0	1.2	1.9	103	92	97	15	1953	1953	250 000	1 000 000	2.1	6	17
Integrin beta-6 (ITGB6)	P18564	2.1	2.6	3.0	123	97	116	15	0.24	0.48	15 625	31 250	4.5	8	17
Interferon lambda receptor 1 (IFN-lambda receptor 1) (IFNLR1)	Q8IU57	2.4	2.8	3.4	80	93	76	15	0.95	1.91	15 625	31 250	3.9	8	17
Interferon regulatory factor 9 (IRF9)	Q00978	0.5	0.7	1.2	154	102	123	15	61	61	250 000	500 000	3.6	23	28
Interleukin-1 receptor-associated kinase 1 (IRAK1)	P51617	0.3	0.9	1.3	81	95	73	15	976	3906	1 000 000	1 000 000	2.4	12	21
Interleukin-1 receptor-associated kinase 4 (IRAK4)	Q9NWZ3	NA	0.8	1.5	NA	NA	NA	15	244	244	1 000 000	1 000 000	3.6	11	18

		Sample types						Endogenous interference	Analytical measurement					Precision	
		Normal plasma levels (NPX)			Relative to EDTA plasma (%)			(mg/mL)	pg/mL				log10	% CV	
Target	UniProt No	10th %tile	Median	90th %tile	ACD	Heparin	Serum	Hemolysate	LOD	LLOQ	ULOQ	Hook	Range	Intra	Inter
Interleukin-10 (IL-10)	P22301	1.7	2.4	3.4	116	94	107	15	15	31	250 000	500 000	3.9	13	14
Interleukin-12 receptor subunit beta-1 (IL-12 receptor subunit beta-1) (IL12RB1)	P42701	1.5	2.0	2.3	98	90	96	15	3.81	7.63	125 000	500 000	4.2	8	19
Interleukin-5 (IL-5)	P05113	NA	0.3	2.1	110	95	103	15	31	61	62 500	125 000	3.0	12	18
Interleukin-6 (IL-6)	P05231	2.0	3.1	4.1	119	97	115	0.2	0.03	0.03	3 906	7 812	5.1	8	14
Islet cell autoantigen 1 (ICA1)	Q05084	NA	NA	0.8	NA	NA	NA	15	1.91	3.81	125 000	250 000	4.5	8	18
Keratin, type I cytoskeletal 19 (KRT19)	P08727	2.0	2.6	4.1	235	93	130	1.9	244	488	1 000 000	1 000 000	3.3	11	12
Leukocyte immunoglobulin-like receptor subfamily B member 4 (LILRB4)	Q8NHJ6	2.5	3.4	4.0	86	94	85	15	61	122	1 000 000	1 000 000	3.9	10	15
Lymphocyte activation gene 3 protein (LAG3)	P18627	1.9	2.3	2.7	118	95	108	0.0	244	244	1 000 000	1 000 000	3.6	7	16
Lymphocyte antigen 75 (LY75)	O60449	1.7	2.3	2.8	118	98	110	15	31	31	31 250	500 000	3.0	9	16
Lysosome-associated membrane glycoprotein 3 (LAMP3)	Q9UQV4	3.3	4.6	5.7	125	98	118	0.5	7.63	7.63	15 625	31 250	3.3	8	19
Mannan-binding lectin serine protease 1 (MASP1)	P48740	1.7	2.4	2.8	110	95	103	15	122	244	125 000	250 000	2.7	6	18
Merlin (NF2)	P35240	NA	NA	NA	NA	NA	NA	15	488	488	31 250	62 500	1.80	11	18
Methylated-DNA--protein-cysteine methyltransferase (MGMT)	P16455	1.6	2.3	4.3	77	97	75	15	122	122	125 000	500 000	3.0	8	16
Natural cytotoxicity triggering receptor 1 (NCR1)	O76036	1.5	2.1	2.6	113	95	107	15	1.91	1.91	31 250	31 250	4.2	8	18
Natural killer cells antigen CD94 (KLRD1)	Q13241	4.8	5.6	6.5	122	97	116	0.0	1.91	1.91	15 625	31 250	3.9	8	16
Neurabin-2 (PPP1R9B)	Q96SB3	1.5	2.6	4.2	68	93	60	15	31	61	125 000	1 000 000	3.3	7	13
Neurotrophin-4 (NT-4) (NTF4)	P34130	1.4	1.8	2.5	93	97	88	15	0.48	0.95	7 812	31 250	3.9	10	17
Nuclear factor of activated T-cells, cytoplasmic 3 (NFATC3)	Q12968	NA	0.4	1.4	NA	NA	NA	15	7.63	7.63	31 250	31 250	3.6	8	17
Parathyroid hormone/parathyroid hormone-related peptide receptor (PTH1R)	Q03431	1.7	2.5	3.5	101	92	98	15	1.91	3.81	7 812	31 250	3.3	9	16
PC4 and SFRS1-interacting protein (PSIP1)	O75475	1.2	1.7	2.5	132	88	113	15	244	244	125 000	250 000	2.7	12	14
Peroxiredoxin-1 (PRDX1)	Q06830	1.0	1.6	2.0	122	96	112	15	31	31	62 500	62 500	3.3	9	13
Peroxiredoxin-5, mitochondrial (PRDX5)	P30044	1.7	2.4	3.4	341	95	229	3.8	244	244	1 000 000	1 000 000	3.6	9	11
Phosphoinositide 3-kinase adapter protein 1 (PIK3AP1)	Q6ZUJ8	1.4	2.4	3.1	135	100	122	15	15	244	125 000	250 000	3.6	13	18
Plexin-A4 (PLXNA4)	Q9HCM2	2.5	3.6	4.7	117	94	110	15	15	31	125 000	250 000	3.6	10	14
Polypeptide N-acetylgalactosaminyltransferase 3 (GALNT3)	Q14435	1.2	1.6	2.1	99	92	95	15	244	976	1 000 000	1 000 000	3	11	17
Probable ATP-dependent RNA helicase DDX58 (DDX58)	O95786	0.8	1.3	2.3	146	95	125	7.5	244	244	1 000 000	1 000 000	3.6	13	22
Protein FAM3B (FAM3B)	P58499	3.0	4.9	5.8	121	95	117	15	15	31	125 000	250 000	3.6	8	15
Protein HEXIM1 (HEXIM1)	O94992	2.3	3.1	3.8	66	99	61	15	0.24	0.48	488	1 953	3.0	6	17
Protein kinase C theta type (PRKCQ)	Q04759	NA	NA	0.6	NA	NA	NA	7.5	488	976	1 000 000	1 000 000	3.0	12	17
Protein sprouty homolog 2 (SPRY2)	O43597	NA	NA	1.7	NA	NA	NA	15	122	244	125 000	125 000	2.7	14	14
Protein-arginine deiminase type-2 (PADI2)	Q9Y2J8	NA	NA	0.7	119	98	103	15	122	244	1 000 000	1 000 000	3.6	10	20
SH2 domain-containing protein 1A (SH2D1A)	O60880	0.7	1.2	1.8	110	96	100	15	15	31	15 625	125 000	2.7	8	15
SH2B adapter protein 3 (SH2B3)	Q9UQQ2	1.0	1.8	3.6	58	98	57	15	122	244	1 000 000	1 000 000	3.6	14	14
Signaling threshold-regulating transmembrane adapter 1 (SIT1)	Q9Y3P8	1.4	2.2	3.2	72	94	69	15	7.63	7.63	7 812	31 250	3.0	9	15
SRSF protein kinase 2 (SRPK2)	P78362	NA	0.5	0.7	NA	NA	NA	3.8	31	31	31 250	31 250	3.0	8	19
Stanniocalcin-1 (STC1)	P52823	5.7	6.6	7.2	86	93	81	15	61	61	15 625	15 625	2.4	7	18
Stromal cell-derived factor 1 (SDF-1) (CXCL12)	P48061	0.6	1.2	1.7	83	93	89	15	244	488	31 250	31 250	1.8	9	19
T-cell-specific surface glycoprotein CD28 (CD28)	P10747	0.5	0.7	1.0	102	90	98	15	7.63	7.63	31 250	62 500	3.6	6	17
Thioredoxin-dependent peroxide reductase, mitochondrial (PRDX3)	P30048	NA	NA	NA	NA	NA	NA	0.5	3906	7812	500 000	500 000	1.8	NA	NA
TNF receptor-associated factor 2 (TRAF2)	Q12933	0.9	1.5	2.1	72	95	65	15	7812	7812	1 000 000	1 000 000	2.1	7	17
TRAF family member-associated NF-kappa-B activator (TANK)	Q92844	NA	0.5	1.6	NA	NA	NA	15	0.95	0.95	62 500	250 000	4.8	11	18
Transcription factor AP-1 (JUN)	P05412	NA	0.4	3.1	117	91	103	15	244	244	500 000	1 000 000	3.3	11	16
Transcription regulator protein BACH1 (BACH1)	O14867	0.4	1.2	1.6	NA	NA	NA	15	15	31	31 250	125 000	3.0	7	16
Triggering receptor expressed on myeloid cells 1 (TREM1)	Q9NP99	1.5	2.0	2.5	145	97	133	15	0.95	1.91	977	1 953	2.7	8	19
Tripartite motif-containing protein 5 (TRIM5)	Q9C035	NA	0.6	0.8	103	NA	102	15	15	31	250 000	500 000	3.9	9	19
Tryptase alpha/beta-1 (Tryptase-1) (TPSAB1)	Q15661	3.1	3.8	4.3	113	89	112	15	31	31	7 812	500 000	2.4	6	16
Tumor necrosis factor receptor superfamily member EDAR (EDAR)	Q9UNE0	0.7	1.0	1.6	149	97	142	15	0.90	0.95	7 812	7 812	3.9	8	16
Zinc finger and BTB domain-containing protein 16 (ZBTB16)	Q05516	NA	0.4	0.9	76	96	79	15	0.95	3.81	31 250	62 500	3.9	10	16

2.3 PRECISION

REPEATABILITY

Intra-assay variation (within-run) was calculated as the mean %CV for 6 individual samples run, within each of 6 separate runs during the validation studies. Inter-assay variation (between-run) was calculated between experiments with the same operator. The reported inter-assay mean %CV is the average of three operators' %CV. Variation calculations were performed on linearized values for 91 analytes for which response levels could be measured in serum and normal plasma, see Table 1.

Across all 91 assays, the mean intra-assay and inter-assay variations observed were 9.3% and 16.8%, respectively. The distributions of intra-assay and inter-assay variations are shown in Figure 4.

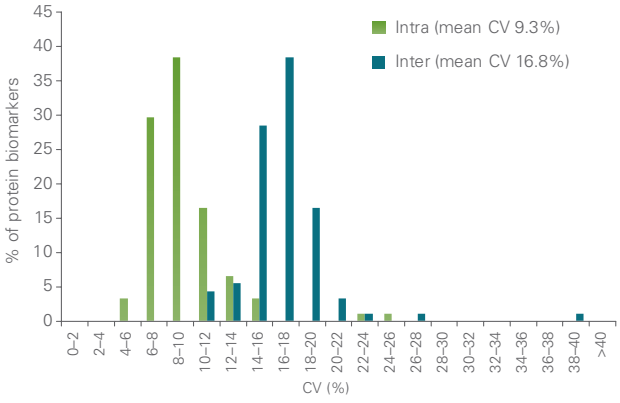

Fig 4. Distribution of intra-assay and inter-assay variations of Olink IMMUNE RESPONSE.

REPRODUCIBILITY

Inter-site variations (between-site) were investigated during the validation of previous panels in beta-site studies to estimate the expected variations in values between different laboratories, with different operators and using different equipment. The beta-site studies have shown reproducibility and repeatability in line with Olink results. For more information, download our Data Validation documents at www.olink.com/data-validation

Olink has Analysis Service labs in Sweden and the USA, and in addition there are many Olink-certified core laboratories around the world running the Olink platform (see www.olink.com/service for details). Our experience over several years is that inter-site reproducibility is very good providing that operators are properly trained. For more information please contact support@olink.com.

2.4 ANALYTICAL SPECIFICITY

ASSAY SPECIFICITY

To test the target-protein specificity of the PEA probes used in the panel, all of the antibodies used were tested for cross-reactivity against all of the recombinant proteins used during assay validation. The probes were also checked for cross-reactivity to more than 100 additional proteins (data not shown). This was caried out by creating a test sample consisting of a pool of antigens, which was then incubated with all 92 antibody probe pairs from the panel. To optimize this analysis, 10 sub-pools of antigen were evaluated to cover the 92 assays (see Figure 5).

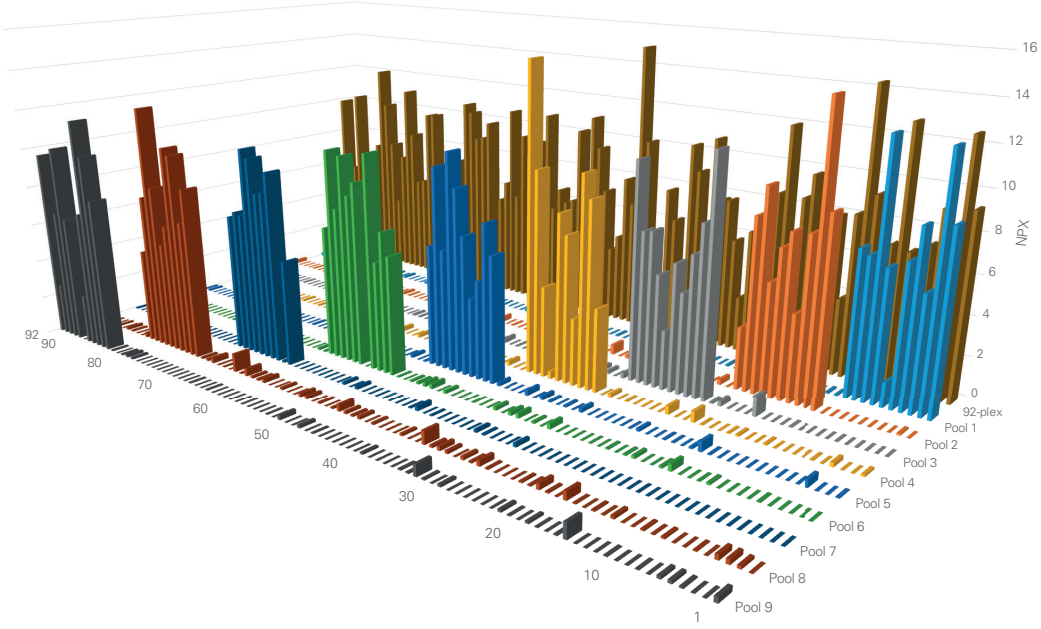

Fig 5. Assay readout specificity of the Olink platform. For each assay, specificity is confirmed by testing antigen sub-pools against the complete 92-plex pool as to each sub-mix.

The lack of significant signal from these tests indicates that each probe pair is specific for its target antigen, demonstrating the readout specificty of the PEA method.

ENDOGENOUS INTERFERENCE

Endogenous interference from heterophilic antibodies, e.g. human anti-mouse antibody (HAMA), and rheumatoid factor are known to cause problems in immunoassays.

To evaluate the potential impact of this specific interference, a special "mismatch" system was designed. The only way to generate a signal in this system is to bring antibody probe pairs into proximity, by cross-binding substances other than antigens, e.g. heterophilic antibodies or rheumatoid factor. No interference due to HAMA or RF could be detected for any of the samples in any of the previously tested panels, indicating sufficient blocking of these agents (data not shown).

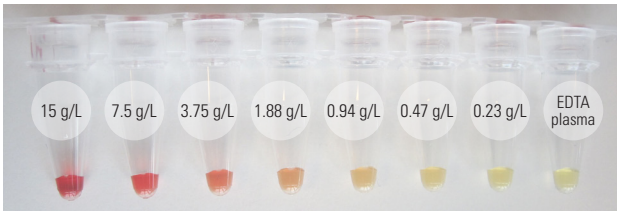

Fig 6. Endogenous interference. Levels tested for hemolysate were 0.23-15 g/L hemoglobin. The highest hemolysate concentration translates to about 10% hemolysis.

The potential impact of some known interfering serum and plasma components was evaluated using serial dilutions of hemolysate, lipids and bilirubin, respectively in EDTA plasma and serum

An example of hemolysate levels tested is shown in Figure 6. These additions simulate different patient health conditions and/or sample collection irregularities. Interference by bilirubin and lipids has previously been evaluated, and disturbance has only been observed at extrem levels corresponding to 8 or 10 times normal [3, 4], values and therefore not performed for Olink IMMUNE RESPONSE. In 22 out of 92 assays, altered values were recorded after the addition of hemolysate. The reason is most likely due to more of the measured analyte leaking out of the disrupted blood cells. A concentration of 15 g/L of hemolysate represents 10% hemolysis of a sample. Table 1 reports the highest concentration of hemolysate that does not have an impact on assay performance.

2.5 SCALABILITY

Assay performance was further evaluated with regard to scalability, meaning the capability of the Olink technology to maintain the same quality of performance irrespective of multiplex grade. Previously, we have shown that a step-wise increase of multiplex grade (8, 24, 48, 72 and 96) does not compromise assay performance (data not shown). To further strengthen that Olink provides consistent results, single assays for Growth Hormone (GH) and Matrix Metalloproteinase (MMP-7) were compared when run in the full Olink CVD II panel. The results for each assay and their observed dCq-values were plotted against the entire 96-plex reaction. The square of the correlation coefficient (R^2) value was generated by linear regression.

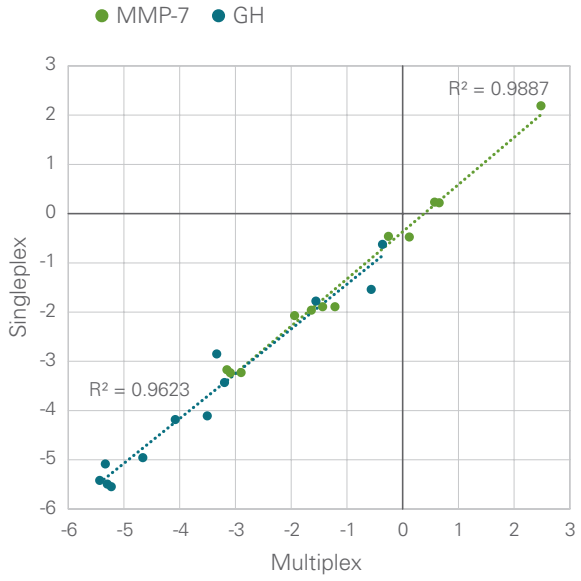

Fig 7. Scalability of the Olink technology platform. This experiment was performed using the Olink CVD II panel. Human plasma samples were analyzed in singleplex for Growth Hormone (GH) and Matrix Metalloproteinase (MMP-7) with the equivalent assays performed in a full 96-plex reaction. The observed dCq (log2) values were plotted, and the correlation coefficient R^2 value was generated by linear regression.

3. References

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TECHNICAL SUPPORT

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